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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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INVENTOR(S)		
GIVEN NAME (first and middle [if any])	FAMILY NAME OR SURNAME	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Gurjit K.	**Hershey**	**Cincinnati, Ohio**
Amal	**Assa'ad**	**Cincinnati, Ohio**
Ranajit	**Chakraborty**	**Cincinnati, Ohio**

[ ] Additional inventors are being named on the ____ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max)

A NOVEL GENETIC MARKER FOR FOOD ALLERGY

CORRESPONDENCE ADDRESS

Direct all correspondence to:

[ ] Customer Number [Type Customer Number Here] → [Place Customer Number Bar Code Label here]

OR

[X] Firm or Individual Name	**Loy M. White**				
Address	**Cincinnati Children's Hospital Medical Center** **Office of Intellectual Property & Venture Development**				
Address	**3333 Burnet Avenue** **MLC 7032**				
City	**Cincinnati**	State	**Ohio**	ZIP	**45229-3039**
Country	**USA**	Telephone	**(513) 636-4285**	Fax	**(513) 636-8453**

ENCLOSED APPLICATION PARTS ***(check all that apply)***

[X] Specification Number of Pages: 30 [ ] CD(s), Number ____

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[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27 [ ] A check or money order is enclosed to cover the filing fees [ ] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-2201 [ ] Payment by credit card. Form PTO-2038 is attached.	FILING FEE AMOUNT: **$80.00**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

[X] No

[ ] Yes, the name of the U. S. Government agency and the Government contract number are: ____

Respectfully submitted,

SIGNATURE ____ Date August 5, 2003

TYPED or PRINTED NAME Loy M. White REGISTRATION NO. 43,262 (if appropriate)

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TOTAL AMOUNT OF PAYMENT **($) 80.00**

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	FEE PAID
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

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			Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		x		=	
Independent Claims		-3** =		x		=	
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Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claims, if not paid
1204	84	2204	42	**Reissue independent claims over original patent
1205	18	2205	9	**Reissue claims in excess of 20 and over original patent

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3. ADDITIONAL FEES

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1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,300	2453	650	Petition to revive – unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Statement	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBMITTED BY

Complete (if applicable)

Name (Print/Type)	Loy M. White	Registration No. (Attorney/Agent)	43,262	Telephone	(513) 636-7512
Signature				Date	08/05/2003

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I hereby certify that this provisional patent application is being deposited with the United States Postal Service, "Express Mail Post Office to Addressee" service, under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Commissioner for Patents, Alexandria, VA 22313-1450.

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PROVISIONAL PATENT APPLICATION

Under 37 C.F.R. § 1.53(c)

A NOVEL GENETIC MARKER FOR FOOD ALLERGY

Gurjit K. Hershey
Cincinnati Children's Hospital Medical Center

Amal Assa'ad
Cincinnati Children's Hospital Medical Center

Ranajit Chakraborty
University of Cincinnati Medical Center

Abstract

Background: Food allergy affects nearly 10% of all individuals. Although the factors important in the pathogenesis of food allergy are not known, there is clearly a genetic predisposition. IL4RA, IL13 and CD14 genetic polymorphisms have been implicated as atopy susceptibility genes by multiple investigators. **Objective:** We examined the genetics of common SNPs in IL4RA, IL13 and CD14 in food allergy to determine if combinations of allelic variants of these genes were important in food allergy. **Methods:** Patients with food allergy were recruited along with non-atopic, non-asthmatic control subjects. Four SNPs in IL-4Rα (I75V, E400A, C431R, Q576R), the R130Q IL-13 SNP and the CD14 -159 C→T promotor polymorphism were genotyped using PCR-based RFLP assays. **Results:** With each locus analyzed at the level of genotypes, the TT (CD14 -159 C→T) genotype was significantly associated with food allergy. However, no significant allele frequency difference between food allergy patients and normal controls was observed at any of the six polymorphic sites, when analyzed individually. Sequential multi-locus analyses revealed significant excess of 2-locus VV (I75V at IL-4Rα) – QR (R130Q at IL-13), and QR (R130Q at IL-13) – TT (at CD14 -159 C→T) in food allergy patients compared to controls (p = 0.029 and 0.011, respectively). This was caused by a dramatic increase of individuals carrying the allele combination of V75IL-4Rα, Q130IL-13, and T-159C→TCD14 in patients with food allergy, compared to controls (p = 0.008). Furthermore, this allele combination was associated with the phenotype of eczema among food allergy patients (p=0.02). **Conclusions:** The V75IL-4Rα/Q130IL-13/T-159C→TCD14 allele combination is strongly associated with food allergy and may be a useful genetic marker to identify at risk infants. Our observations also demonstrate that it is critical to study the effects of common genetic variants in combination since the significance of a given

SNP may only be evident in individuals carrying additional specific variant alleles in the same or different genes.

Key words: Food Allergy, Genetics, Toll Receptors, Cytokine, Cytokine Receptor

Introduction

Food allergy is a common condition, with an estimated prevalence of 8% in the pediatric population and 2% in the adult population. (1). Food allergy can be life-threatening and the primary therapy is avoidance of the allergenic food. Although the mechanisms underlying the pathogenesis of food allergy are unclear, food allergy has a familial occurrence (2, 3) supporting that there is a genetic predisposition. Although considerable effort is taking place to identify the genes important in the development of atopy, very little has been done in the area of food allergy. Studies have demonstrated associations between Class II HLA genotypes and food allergy (4, 5). Furthermore, there is some data that food allergy may be associated with a slow acetylation genetic variant of N-acetyltransferase 2 (6). Since food allergy occurs with higher frequency in subjects with other atopic disorders including atopic dermatitis (7, 8) asthma (9, 10) and allergic rhinitis (11), some of the genes shown to be associated with atopy may be relevant in food allergy. IL-4 and IL-13 are Th2 cytokines that are critical for the development of allergic inflammation. They both exert their effect through a common receptor, IL-4 receptor alpha (IL-4Rα). Genetic variants of IL-13 and IL-4Rα have been associated with atopy by multiple studies (12-14). IL-4 and IL-13 have also been shown to be important in food allergy. T cell clones specific to ovomucoid derived from egg-allergic subjects consistently expressed interleukin (IL)-5. IL-4, IL-13 (15). Furthermore, a marked increase in IL-4 synthesis by peripheral blood lymphocytes was observed following *in vitro* stimulation with ovalbumin in patients with active atopic dermatitis and egg sensitivity; in contrast, cells from similar patients with atopic dermatitis in remission displayed decreased IL-4 synthesis levels comparable to that seen in normal individuals (16). In addition, peripheral blood mononuclear cells derived from

patients with food allergy displayed significantly increased IL-4 production following food challenge compared with prior to challenge (17).

Despite the similarities of food allergy with other atopic disorders, several characteristics of food allergy are quite distinct. Food allergy often presents very early in life, during the first few days or weeks of life while IgE responses are still immature. Thus, unlike other atopic disorders, food allergy is likely to be less dependent on mechanisms involving IgE. Furthermore, while sensitization to environmental allergens requires previous exposure of at least several years, sensitization to food allergens may occur after only one exposure or even in the absence of previous exposure (18, 19). The early onset of food allergy points towards a role for innate immunity in its development. CD14 is a pattern recognition receptor that binds lipopolysaccharide (LPS) as well as other bacterial components. Engagement of CD14 by LPS results in activation of antigen presenting cells including macrophages and dendritic cells, and subsequent release of proinflammatory cytokines and mediators (20). Recently, the gene for CD14 has been reported to contain several polymorphisms its coding and promoter regions. One polymorphism, a C to T transition at position -159 (-159 C→T), has been shown to associate with atopy (21, 22). However, a more recent study found no association of this CD14 SNP with asthma, atopic dermatitis, allergic rhinitis, total or specific IgE levels (23).

We recently found that the T allele, and especially the TT genotype, of the CD14, -159C→T polymorphism was significantly associated with food allergy (24). Since innate factors as well as IL-4 and IL-13 have been shown to be important in food allergy, we examined the genetics of IL-4 receptor alpha, IL-13 and CD14 in a group of patients with IgE-mediated food allergy. We focused on SNPs in each gene that have been shown to have functional consequences. Previous studies have demonstrated a complex interaction between the anti-

inflammatory, atopy-related cytokines, IL-13 and IL-4 and the levels of expression of CD14 on mononuclear cells and in the soluble form (25, 26). We reasoned that a combination of genes important in innate immunity and the development of Th2 immunity and IgE may act together to promote the food allergy phenotype.

Methods

Study subjects

Patients were recruited sequentially from the food allergy clinic at Cincinnati Children's Hospital Medical Center. Patients were also recruited from the office of a private community allergist and from an advertisement to the local food allergy support group. Patients were offered to participate in the study if they had a confirmed diagnosis of food allergy. This was defined as a history of an immediate adverse reaction to a food with objective symptoms and signs and a positive prick skin test and/or RAST to the food. Food challenges were done as indicated by the clinical history. A positive food challenge was taken as further confirmation of the diagnosis. The following information was collected during the clinical evaluation by a physician and/or from a questionnaire completed by the patient, or their caregiver(s) in the case of pediatric age patients: (1) A list of the food/s that has/have caused an adverse reaction; For each food listed, the following information was gathered: the age at which the first reaction occurred, a description of the reaction, the time between ingestion of the food and the onset of the reaction, whether the reaction was evaluated by a physician immediately or later, the method used to establish the diagnosis whether by prick skin tests and/or RAST, whether a food challenge was performed, whether the food had subsequently been eliminated from the diet, and whether the food was reintroduced in the diet any time later; (2) any co-existing allergic diseases, such as asthma or eczema. Eczema was diagnosed by history (a definite history of dry scaly skin and use of topical moisturizers and/or topical steroids) and/or physical exam. Because of the predominantly young age of the patient population and the difficulty in establishing the diagnosis of asthma in young patients, the diagnosis of asthma was not included in the analysis. Food reactions were classified as mild or severe according to the following definition. A severe

reaction was defined as generalized anaphylaxis or a reaction that caused respiratory compromise, including angioneurotic edema endangering the airway. Cutaneous reactions and swelling of other parts of the face, e.g. the eyes, and mild vomiting were considered mild reactions.

Healthy, non-allergic, non-asthmatic, unrelated subjects were prospectively recruited from University of Cincinnati Medical Center and the Cincinnati Children's Hospital Medical Center. Individuals were excluded from this group if they reported a history of allergies, including food allergies, asthma, chronic cough or COPD. The subjects underwent skin prick tests including positive and negative controls, and a panel of 14 common environmental antigens indigenous to the Ohio Valley (A.L.K. Laboratories Inc., Wallingford, CT). Subjects who demonstrated no positive skin prick tests, other than to the histamine, were included in the control group.

These studies were approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board.

Skin prick tests

Skin prick tests were done to the food(s) that caused the initial adverse reaction(s), as well as to any other foods that have caused suspected adverse reactions. Skin prick tests were performed using commercially available extracts (Hollister Steer Laboratories, Spokane WA and Greer Laboratories, Lenoir, NC) at a concentration of 1:10, 1:20 or 1:40 according to the manufacturer's instruction for each food extract. Histamine 1 mg/cc was used for a positive control, and albumin saline was used as a negative control. The tests were read after fifteen minutes and interpreted by comparing the size of the wheal and flare to the positive and negative control as follows: 0=same size as negative control; 1+ = very small induration, erythema

present; 2+ = 50% of histamine control; 3+ = same as histamine control; 4+ = >histamine control or pseudopodia. Prick skin tests graded as 2+ or higher were considered positive. RAST tests were done at a commercial laboratory. RAST scores of 2 or higher were interpreted as positive.

Food challenge

Food challenges were performed on subjects who had no history of anaphylaxis to the food in question after consent for the challenge was obtained. Challenges were performed as clinically indicated. They were performed either at baseline to establish the diagnosis, or were timed to establish resolution of the food allergy after a period of follow up. Given the predominantly pediatric age of our population, open challenges were performed by administering gradually increasing amounts of the food given at observed intervals dictated by the history (usually every 15 minutes). The challenge was performed on an empty stomach. A challenge was considered positive if any of the following objective signs were noted during the challenge or immediately after its completion: dermatologic signs, e.g. skin rash or edema; gastrointestinal, e.g, vomiting; or respiratory signs, e.g, sneezing, stridor or wheezing.

Genotyping of IL-4Rα, CD14, and IL-13 SNPs

Genomic DNA was isolated from EDTA anti-coagulated whole blood or from a buccal swab by methods described in Risma et al. (27). The IL-4Rα variants were genotyped as previously described (27). We used the following primers: sense 5'-GTGCCAACAGATGAGGTTCAC-3'; and antisense 5'-GCCTCTGACAGTTTATGTAATC-3' to determine the genotypes at the -159 C→T CD14 SNP site. These primers amplified a 497 bp segment of the CD14 promoter from -517 to -19. An *AvaII* restriction site exists at position -159, such that the T allele is cut, resulting in bands of 144 and 353bp, while the C allele remains uncut at 497 bp. After PCR amplification, the reaction volume was digested with 10U of *AvaII*

(New England Biolabs, Beverly, MA) and restriction fragments were resolved on a 2% agarose gel. The IL-13 R130Q variant was genotyped as previously described (14).

Statistical Analyses

Conformity of genotype frequencies at each of the six sites with their respective Hardy-Weinberg expectations was tested by the goodness-of-fit chi-square test (28). Genotype/allele frequency differences between patients and controls were tested by the r by c contingency table test, with levels of significance empirically determined by the permutation test (29). We used 10,000 replications of permutation for each of these tests.

Multi-locus association for food allergy was tested in two steps. First, allele combinations of 6 SNP sites showed that all observed genotypes are explained by 24 allele combinations (of the possible $2^6 = 64$), 8 of which explained 72% of the genetic diversity in food allergy patients, and 79% in the controls. These 8 allele combinations determined by genotypes at three sites I75V at IL-4Rα, R130Q at IL-13, and C→T 159 – CD14, prompting investigation of food allergy associations involving three pairwise combinations of loci (I75V at IL-4Rα and R130Q at IL-13, I75V at IL-4Rα and CD14 -159 C→T, and R130Q at IL-13 and CD14 -159 C→T) and one 3-locus combination (I75V at IL-4Rα, R130Q at IL-13, and CD14 -159 C→T). In the second step, for each of these tests, again, permutation-based levels of significance (with 10,000 replications) were determined for the respective r by c contingency tables. Finally, genotype-combinations exhibiting significant frequency differences between food allergy patients and controls were examined to determine the specific set of allele combinations (at 2- and 3-locus levels) that explained the multi-locus genotype association with food allergy.

For the genotype:phenotype association analyses, the number of individuals carrying the specific combination of alleles was counted by classifying individuals into different

categories of phenotypes. Frequency differences in such categorical data were tested by the same r by c contingency table test, used earlier. In the case when the phenotypes could be nominally ordered (e.g. age at first reaction), the trend test (30) was used to determine whether or not the proportion of patients carrying the specific 3-locus allele combination increases with lower age of onset of allergy.

Results

Patients

One hundred and ten patients with food allergy and 82 non-atopic control subjects were included in this study. The demographic characteristics of the study participants are shown in Table I. The racial distribution of the population mirrors that of the Greater Cincinnati area from which patients and controls were drawn (2000 Census figures for Cincinnati Metropolitan Statistical Area, www.censusscope.org, accessed 2/11/2003). Table 2 outlines the characteristics of the subjects with food allergy. All patients had a diagnosis of IgE-mediated food allergy as defined in the Methods section. Of the 110 patients, 101 patients had a history of immediate adverse reaction to one or more foods and a positive prick skin test to one or more foods. Sixty-six of these patients also had positive RAST to one or more foods. Nine patients had a history of immediate adverse reaction to one or more foods that was confirmed by RAST only. In very few instances, particularly with peanut allergy, the diagnosis of an allergy to peanuts was made in the absence of a history of ingestion in a patient already had an established diagnosis of food allergy to a different food. This was based on a positive skin test and/or RAST to peanuts, which were in the range that would predict a positive ingestion challenge. Forty-three patients had one or more food challenges with a total of 67 food challenges performed. Thirty-three of these patients (77%) had a clinical reaction to one or more food with a total of 47 positive food challenges.

The mean age at study entry was 6.3 years $\pm$ 8.2, and ranged from 0.48 to 42.8 years, with 30% of the patients being less than 2 years of age and a total of 93.6% of patients of pediatric age (<18 years). The age of the subject at the time of the first food allergy reaction (obtained from the history) paralleled the age at study entry with 84.5% of the first food allergy reactions occurring in patients less than 2 years of age.

There was a high prevalence of eczema in the patient population. Of 99 patients in whom the information was available, 54.5% had a diagnosis of eczema. Almost half the patients had an allergy to only one food, and about a third had an allergy to two foods. The patients had a total number of 208 food allergies. Of these, 28% were caused by peanuts, 18% by cow's milk, 18% by eggs, 6% by fish, 5% by soy and 4% by wheat. Additional foods that occurred with lower frequency included tree nuts, beef, chicken, pork, lobster, shrimp, lentils, strawberries and cantaloupe.

Analysis of IL-4Rα, IL-13, and CD14 SNPs in subjects with food allergy

We genotyped the individuals with food allergy and the control subjects for 6 SNP sites in 3 different genes, IL-4RA (I75V, E400A, C431R, and Q576R), IL-13, and CD14. As shown in Table 3, all of the genetic markers were in Hardy-Weinberg equilibrium except for the IL-4Rα E400A in the cases ($p=0.015$). Genotype frequencies of the CD14 -159C→T site were significantly different between cases and controls ($p=0.036$). This was due to an increase of the CD14 TT genotype in patients with food allergy with the odds ratio of 3.523 (95% CI: $1.265<OR<8.369$) confirming our previous findings (24).

A specific combination of IL-4Rα, IL-13, and CD14 alleles is a genetic marker for food allergy

The above observation of an increased frequency of the TT genotype at the CD14 –159 C→T locus in patients with food allergy implicates CD14 in the genetics of food allergy. However, food allergy is a complex multi-factorial disease so it was important to examine multiple genes. Also, the association of CD14 is not significant at the level of alleles (i.e., "C" vs. "T" frequency difference, $p = 0.118$). With the awareness of this, we first examined the allele combination of all six SNP sites, showing that 8 out of 24 allele combinations can explain 72% of the genetic diversity in patients of food allergy, and 79% in normal controls. These 8 allele

combinations are determined by genotypes at three sites I75V at IL4Rα, R130Q at IL13, and 159 C→T at CD14. Then, investigation of food allergy association was conducted focusing on these SNPs at the 2-locus level and 3-locus level respectively. At the 2-locus level, frequencies of genotype VVQR (between I75V at IL4Rα and R130Q at IL13) and genotype QRTT (between R130Q at IL13 and 159 C→T at CD14) were significantly higher in patients with food allergy than normal controls with p value of 0.029 and 0.011 respectively as shown in Table 4, odds ratio for genotype VVQR is 4.109 (95% CI: 1.161<OR<14.537), and for genotype QRTT is 10.263 (95% CI: 1.323<OR<79.618). At the 3-locus level, we observed 8 individuals carrying the genotype of VV (I75V at IL4Rα)-QR (R130Q at IL13)-TT (CD14) in patients with food allergy, and 0 in normal controls (p = 0.054). We further investigated whether the combination of these three loci associated with food allergy (Table 5). Two allele combinations can be derived from genotype VV-QR-TT, one is V-Q-T, and the other is V-R-T. The number of individuals carrying this specific allele combination V-Q-T was 33 among patients with food allergy versus 8 in the normal control group (p = 0.008, odds ratio is 3.107, 95% CI: 1.336<OR<7.228). The number of individuals carrying the other allele combination of V-R-T was not significantly different between patients with food allergy and controls. These results support that the specific allele combination of V (I75V at IL4Rα)-Q (R130Q at IL13 –T (CD14) constitutes the major genetic predisposition underlying food allergy investigated in our study. Importantly, the combination of genetic variants in all 3 genes was far superior to a single gene or even to 2 genes together. The Q576R IL-4Rα allele has been shown to be strongly associated with atopy and atopic asthma by our group and others (27, 31-33). We specifically examined whether the effect of this allele was significant in combination with any combination of the other allelic variants of IL-4Rα, IL-13 and CD14 in food allergy, but found no significant associations.

Genotype:Phenotype relationships between the V75IL-4Rα/Q130IL-13/T-159C→T combination and food allergy

We next explored whether there were any significant genotype:phenotype relationships among the food allergy patients. The number of individuals carrying the specific combination of alleles was counted by classifying individuals into different categories of phenotypes (Table 6). The presence of the specific allele combination was significantly associated with eczema ($p = 0.010$). Patients with food allergy and eczema ($n = 53$) had a prevalence of 41.5% carrying the specific allele combination, which is significantly higher when compared to 17.7% in patients with food allergy without eczema ($p = 0.017$). Thus, the association of the allele combination is even stronger among patients with food allergy and eczema. For the phenotype "age at first reaction", we conducted a trend test (30) to examine whether the frequency of patients carrying the V75IL-4Rα/Q130IL-13/T-159C→T combination increases with a lower age at the time of the first food allergy reaction. We observed that the proportion of patients carrying the specific allele combination was significantly increased among patients with a lower age of onset of food allergy ($p = 0.018$). No significant relationship was noted between the individuals carrying the specific allele combination and the number of food allergies, reaction severity, or a particular food. However, larger studies will be necessary to adequately address these relationships.

Discussion

Food allergy has a genetic predisposition but very little is known about the genetics of food allergy. Herein, we demonstrate for the first time that a specific combination of allelic variants in 3 different genes, CD14, IL-4RA, and IL-13, was strongly associated with food allergy. Specifically, the combination of the V75 allele of the IL-4Rα, coupled with Q130 IL-13 and the T allele of −159C→T of CD14 alone was a genetic marker for food allergy. All 3 of these genetic variants have been shown to be functionally relevant, resulting in enhanced expression (34) or activity (27, 35) of their respective gene products. Importantly, the combination of these three variant alleles exhibit a much stronger association with food allergy than any of these individual alleles. At a single site level, significant association of the T −159 C→T CD14 allele, and especially of the TT genotype with food allergy was observed, confirming our previous observation (24). However, the combination of this allele with the IL-4Rα and IL-13 atopy-associated variants resulted in a much stronger significant association.

This observation is of wider significance because it illustrates the importance of examining the consequences of SNPs in multiple genes in combination. A given SNP may only be relevant in the context of a second or a combination of additional SNPs in the same gene or other genes. We recently showed that this was indeed the case for a combination of IL-4Rα allelic variants (27). Furthermore, a given SNP may have no effect individually or in combination with a different set of SNPs. Genetic association studies have often been difficult to interpret due to poor reproducibility in other populations. One reason for this may be that a given genetic variant may not be important unless it is examined in the context of one or more additional SNPs. This would contribute to disparate results in different populations.

Since IL-4Rα is also a necessary signaling component of the IL-13 receptor complex, it is not surprising that atopy-associated genetic variants in these 2 genes (IL-13 and IL-4RA) may act in a concerted fashion. This is supported by the recent report demonstrating interactive genetic effects between SNPs in IL-4Rα and the IL-13 promotor (36). However, the mechanisms underlying the association of the IL-13/IL-4Rα pathway with CD14 is less obvious. Interactions between CD14 and IL-4 have been described. While bacterial products such as LPS activate monocytes and increase CD14 expression, IL-4, which has antiinflammatory properties, downregulates CD14 expression. An LPS-induced increase in CD14 expression rescued monocytes from apoptosis, whereas IL-4 treatment resulted in decreased CD14 expression and eventual apoptosis. Down-regulation or removal of CD14 triggers apoptosis, whereas up-regulation promotes survival of monocytes (25). Interactions between CD14 and IL-13 have also been reported. Similar to the previously described effect of IL-4, IL-13 down-regulates CD14 by suppressing CD14 RNA expression (26). Furthermore, in a segmental antigen challenge model, soluble CD14 levels correlated with IL-13 concentrations 18 h after the challenge (37). Thus, IL-4 and IL-13, which both signal via IL-4Rα, have direct effects on CD14 expression, and this may contribute to the mechanism by which genetic variants of these 3 genes act together to promote food allergy.

The roles of membrane vs. soluble CD14 in atopic diseases remain unclear. A complex of LPS and LPS-binding protein in the serum initiate signals in monocytes and macrophages via membrane CD14. However, soluble CD14 can also initiate signals, thus cells lacking CD14 can also respond to LPS. LPS, acting through its receptor complex including CD14, TLR4, and MD-2 (20) induces maturation of antigen presenting cells (APC) including dendritic cells and macrophages. Increased CD14 expression would result in increased LPS binding and increased

production of proinflammatory mediators by monocytes and macrophages including prostaglandins, reactive oxygen and nitrogen intermediates, IL-1, IL-6, IL-8, TNFα, and IL-12 (20). Recently, the T allele was shown to result in approximately 32% increased transcriptional activity of the promoter when compared with the C allele and the mechanism of this association was related to alterations in transcription factor binding to this region (38). Thus, this SNP may be associated with alterations in CD14 expression, and the relative ratios of soluble vs. membrane CD14. Studies have found that soluble CD14 levels are higher in asthmatics than non-asthmatic controls (39). Similarly, we find that the T allele, which is associated with increased transcriptional activity, has increased frequency among subjects with food allergy.

The more important question is why does an allele associated with an increase in transcription of CD14 correlate with the presence of food allergy, especially when combined with functionally relevant genetic variants of IL-13 and IL-4Rα? The mechanism for this association may be that the LPS in foods acts essentially as an adjuvant and increases the specific IgE response to foods in susceptible individuals. The IL-13 and IL-4Rα allelic variants would contribute to the IgE response. Alternatively, an increased pro-inflammatory response in the gut secondary to the presence of the T CD14 allele, increased CD14 production, and resultant increased inflammatory cytokine production, may be associated with an enhanced sensitization to food allergens, especially early in life. Finally, the association may be the result of direct interplay between IL-4, IL-13 and CD14 that determines the final contribution of CD14 pathways by modulating total as well as soluble vs. membrane CD14 levels.

The clinical utility of the food allergy associated haplotype identified herein remains to be seen. Prospective studies will be necessary to examine the predictive value of this haplotype in predicting food allergy. Early identification of at-risk infants has obvious potential advantages

including earlier initiation of therapeutic interventions resulting in possible attenuation or delay in the phenotype. Alternatively, the genetic marker may serve to identify certain phenotypes among food allergy patients. For example, our data demonstrates a significant association with atopic dermatitis among patients with food allergy. Food challenge remains the gold standard for diagnosis of food allergy. This test, albeit very informative, is not without serious risks and potential complications. The genetic marker we have identified may serve to identify a subgroup of patients with a specific pattern of response to a food, and may predict positive challenges, making food challenge unnecessary in some cases. Furthermore, the combination genotype may be predictive of a specific natural history or response to therapy and aid in the management of food allergy. Large prospective studies will be necessary to adequately address these possibilities. Although important questions remain, our data identifies a novel genetic marker of food allergy and provides novel insights into the pathogenesis of food allergy.

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Table 1. Demographic characteristics of Food allergy cases and Non-atopic control populations

Population		Food allergy cases (n=110)	Non-atopic controls (n=66)
Age ± SD [range]		6.3 ± 8.2 [0.48, 42.8]	30.3 ± 7.7 [20, 54]
Sex	Male	72.7%	53%
	Female	27.3%	47%
Ethnicity	Caucasian	81.8%	83.3%
	African-American	12.7%	4.5%
	Asian	2.7%	6.1%
	Mixed	0.9%	0%
	Other	0.9%	3.03%
	Unknown	0%	3.03%

Table 2: Characteristics of Food Allergy Subjects

Age at first reaction (Mean ± SD)	Number of subjects
Age < 1 year (0.7 ± 0.2)	58
1-2 years (1.7 ± 0.3)	18
2-18 years (7.6 ± 4.3)	11
Age >18 years (31 ± 3.6)	3
TOTAL	90

Association with eczema	Number of subjects
With eczema	53
Without eczema	45
Not known	12
TOTAL	110

Number of food allergies	Number of subjects
1	53
2	31
3	14
4	9
5	3
TOTAL	110

Food causing allergy	Number of subjects
Peanut	59
Milk	38
Egg	37
Fish	13
Soy	10
Wheat	8

Type of reaction	Number of subjects
Mild	71
Severe	39
TOTAL	110

Table 3. Genotype Frequency of IL-4Rα, IL-13 and CD14 polymorphic variants in Individuals with Food Allergy and Non-atopic Controls

Marker	Food Allergy Cases (n = 110)	Non-atopic Controls (n = 66)	P value
I75V IL-4Rα	II: 26 (0.236) IV: 56 (0.509) VV: 28 (0.254) -- HWE: p = 0.846	II: 17 (0.258) IV: 38 (0.576) VV: 11 (0.167) -- HWE: p = 0.191	0.400
E400A IL-4Rα	EE: 89 (0.809) EA: 17 (0.154) AA: 4 (0.036) -- **HWE: p = 0.015***	EE: 54 (0.818) EA: 11 (0.167) AA: 1 (0.015) -- HWE: p = 0.618	0.797
C431R IL-4Rα	CC: 96 (0.873) CR: 13 (0.118) RR: 1 (0.009) -- HWE: p = 0.463	CC: 54 (0.818) CR: 12 (0.182) RR: 0 -- HWE: p = 0.417	0.412
Q576R IL-α	QQ: 65 (0.591) QR: 36 (0.327) RR: 9 (0.082) -- HWE: p = 0.222	QQ: 43 (0.652) QR: 20 (0.303) RR: 3 (0.045) -- HWE: p =0.732	0.566
R130Q IL-13	RR: 62 (0.564) RQ: 45 (0.409) QQ: 3 (0.027) -- HWE: p = 0.119	RR: 46 (0.697) RQ: 17 (0.258) QQ: 3 (0.045) -- HWE: p = 0.394	0.117
-159 C→T CD14	CC: 36 (0.327) CT: 47 (0.427) TT: 27 (0.245) -- HWE: p = 0.143	CC: 23 (0.348) CT: 37 (0.561) TT: 6 (0.091) -- HWE: 0.103	**0.036*** (for TT: p = 0.015*)

HWE= Hardy Weinberg Equilibrium

Table 4. Genotype frequency difference in food allergy patients and controls at 2-locus level.

I75V IL-4Rα and R130Q IL-13			R130Q IL-13 and CD14 159C_T		
Genotype	Freq. in Cases	Freq. in Controls	Genotype	Freq. in Cases	Freq. in Controls
IIQQ	1 (0.009)	1 (0.015)	QQCC	2 (0.018)	0 (0.000)
IVQQ	2 (0.018)	2 (0.030)	QRCC	10 (0.091)	8 (0.121)
VVQQ	0 (0.000)	0 (0.000)	RRCC	24 (0.218)	15 (0.227)
IIQR	4 (0.036)	6 (0.091)	QQCT	1 (0.009)	3 (0.045)
IVQR	23 (0.209)	8 (0.121)	QRCT	20 (0.182)	8 (0.121)
VVQR	**18 (0.164)**	**3 (0.045)**	RRCT	26 (0.236)	26 (0.394)
IIRR	21 (0.191)	10 (0.152)	QQTT	0 (0.000)	0 (0.000)
IVRR	31 (0.282)	28 (0.424)	**QRTT**	**15 (0.136)**	**1 (0.015)**
VVRR	10 (0.091)	8 (0.121)	RRTT	12 (0.109)	5 (0.076)
P-value*			P-value*		
overall	0.073		overall	0.024	
specific	genotype VVQR: 0.029		specific	genotype QRTT: 0.011	

* P-value is obtained by 10,000 replicates of permutation.

Table 5. 3-locus genotype frequency difference in food allergy patients and controls at 3-locus level.

3-Locus*	Cases		Controls	
VV-QR-CC	3	(0.027)	1	(0.015)
VV-QR-CT	7	(0.064)	2	(0.030)
VV-QR-TT	**8**	**(0.073)**	**0**	**(0.000)**
VV-RR-CC	2	(0.018)	2	(0.030)
VV-RR-CT	5	(0.046)	5	(0.076)
VV-RR-TT	3	(0.027)	1	(0.015)
IV-RR-CC	12	(0.109)	9	(0.136)
IV-RR-CT	13	(0.118)	16	(0.242)
IV-RR-TT	6	(0.055)	3	(0.046)
IV-QQ-CC	1	(0.009)	0	(0.000)
IV-QQ-CT	1	(0.009)	2	(0.030)
IV-QR-CC	6	(0.055)	4	(0.061)
IV-QR-CT	11	(0.100)	4	(0.061)
IV-QR-TT	6	(0.055)	0	(0.000)
II-QQ-CC	1	(0.009)	0	(0.000)
II-QQ-CT	0	(0.000)	1	(0.015)
II-QR-CC	1	(0.009)	3	(0.046)
II-QR-CT	2	(0.018)	2	(0.030)
II-QR-TT	1	(0.009)	1	(0.015)
II-RR-CC	10	(0.091)	4	(0.061)
II-RR-CT	8	(0.073)	5	(0.076)
II-RR-TT	3	(0.027)	1	(0.015)

P-value[&]

overall 0.325

specific genotype VV-QR-TT: 0.054

* These 3 loci are: I75V at IL-4Rα, R130Q at IL-13, and CD14 -159 C→T.

[&] P-value is obtained by 10,000 replicates of permutation.

Table 6. Association of V75 IL-4Ra/Q130 IL-13/T –159C→T CD14 Genetic Haplotype with Food Allergy Phenotypes

Association with eczema	N	Number (%) of subjects with haplotype	p value
Controls	66	0 (0%)	**p<0.0001**
Food Allergy patients	110	33 (30%)	

Age at first reaction (Mean ± SD)	N	Number of subjects with haplotype	p value
Age < 1 year (0.7 ± 0.2)	58	20 (34.5%)	p=0.187
1-2 years (1.7 ± 0.3)	18	5 (25%)	
2-18 years (7.6 ± 4.3)	11	1 (9.09%)	
Age >18 years (31 ± 3.6)	3	0 (0%)	

Association with eczema	N	Number of subjects with haplotype	p value
With eczema	53	22 (41.5%)	**p=0.020**
Without eczema	45	8 (17.7%)	

Number of food allergies	N	Number of subjects with haplotype	p value
1	53	18 (33.9%)	p=NS
2	31	8 (25.8%)	
3	14	4 (28.6%)	
4	9	3 (33.3%)	
5	3	0 (0%)	

Food causing allergy	N	Number of subjects with haplotype	p value
Peanut	59	21 (35.6%)	p=NS
Milk	38	12 (31.6%)	
Egg	37	13 (35.1%)	
Fish	13	3 (23.1%)	
Soy	10	0 (0%)	
Wheat	8	1 (12.5%)	

Type of reaction	N	Number of subjects with haplotype	p value
Mild	71	25 (35.2%)	p=0.164
Severe	39	8 (20.5%)	

NS=not significant

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